Remarks

Claims 1, 2, 5, 7-9, 11, and 21-27 are currently pending. New claims 28-37 directed to the elected invention are submitted herewith. Claims 7, 8 and 21-23 are presently amended to more clearly define the claimed methods. The new claims and amendments are supported by the Applicant's specification, and in the priority document. No new matter has been added.

This response is being filed with a request for a one-month extension of time, and fees associated therewith. If there are any additional fees due in connection with the filing of this response, please charge the fees to our Deposit Account No. 50-0573, and please grant any additional extension of time as may be required.

The Claims are Not Anticipated under 35 U.S.C. § 102(b) by Kreutzer

Claim 22, and claims 24-27 dependent thereon, stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by the teachings of Kreutzer *et al.* (WO 00/44895) ("Kreutzer"). Applicant respectfully traverses the rejection.

Applicant thanks the Examiner for acknowledging that the Declaration of Alan Gewirtz effectively antedates the reference for purposes of 35 U.S.C. § 102 for the claims previously rejected thereunder. The remaining rejection, now made under 35 U.S.C. § 102(b), appears based on the fact that claims 22 and 23 did not receive the benefit of the priority date of the provisional application, 60/248,346, because the provisional application allegedly did not teach "small interfering guide sequences" as claimed.

Claim 22 has been amended and no longer recites "small interfering guide sequences." The claims as amended are directed to methods for disrupting expression of a mammalian target gene *in vitro* in a human cell, wherein the method comprises providing an RNA sequence homologous to a portion of the target gene, said RNA capable of inducing RNAi of the target gene. Support for the amendment can be found, for example, on pages 2-4 of the priority document. Applicant respectfully requests that the amended claim, and those dependent thereon, be granted the benefit of the earlier priority date. Such date effectively makes Kreutzer unavailable as prior art under 35 U.S.C. § 102(b).

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection under 35 U.S.C. § 102(b).

The Claims are Not Anticipated by Fire *et al.*

Claims 1, 2, 5, 7-9, 11, 21-22, and 24-27 stand rejected under 35 U.S.C. §102(e) as allegedly anticipated by Fire *et al.* (United States Patent No. 6,506,559) ("Fire") for the reasons of record as stated in the Office Action mailed 5/23/05.

Applicant continues to traverse the rejection.

As a preliminary matter, Applicant thanks the Examiner for the opportunity to clear the record regarding his comments that Applicant was allegedly "clearly denigrating the validity of claimed subject matter belonging to Fire *et al.*, since Fire *et al.* has patented claims directed to the instant invention in animal cells, and since applicants assert that Fire is enabled only for RNAi in *C. elegans*." For the record, Applicant notes that neither the Applicant, the Applicant's representatives, nor the representatives' law firm have any reason to state an opinion as to the validity of Fire's issued patent. Fire's patent speaks for itself. Rather, Applicant's remarks, and arguments, including those made through its counsel are intended to address the inability of the Fire patent to enable a skilled practitioner to practice *Applicant's* claims. Applicant's representatives are not only entitled to, but are legally obligated to, provide zealous advocacy. Establishing a factual record regarding any prior art that is cited during prosecution as allegedly anticipatory or part of a *prima facie* case of obviousness comes under that duty. Thus, it is for the PTO or a court to decide whether Fire's own claims are valid or invalid, enabled or not enabled, should the question come before them. Applicant's remarks regarding Fire's extent of "enablement", and the factual record set forth in that vein, are intended to establish that the Fire patent is not a novelty-defeating reference under 35 U.S.C. § 102, because it would not have enabled the skilled artisan to practice the Applicant's claims at the time of its (Fire's) filing. This is not only proper, but required of Applicant's representative.

Moving on to substantive matters, as a courtesy to the new examiner on the case (the third examiner who has handled the prosecution of the instant application), Applicant provides herein a brief review of the prosecution and relevant law. The following summary is supplemented by the references themselves attached hereto as Exhibits 1, 2, 3, 4, and 5 (see below), as it is clear from the Office Action that the new examiner has not had, but would like, the opportunity to review the references supplied to the previous examiner. It is also not clear if

each of these references is present in the electronic file wrapper, although at least several are currently available on PAIR.

Fire does not enable the skilled artisan to practice the claimed methods without undue experimentation, and thus is not an anticipatory reference under 35 U.S.C. § 102.

The rejection of claim 1 and claims dependent thereon under 35 U.S.C. § 102 appears to rely heavily on the generalized "teachings" of the Fire patent, including the broadly-construed claims. The rejection relies heavily, as well, on the presumption of enablement of an issued U.S. patent. Applicant has repeatedly traversed this rejection in his responses, on the grounds that the Fire patent does not teach, or in the alternative, does not enable a skilled artisan to practice, each and every limitation of Applicant's claimed method. The pending Office Action, repeats, virtually unchanged, a rejection first made in the Office Action of 10/6/2004, despite the evidence of record.

"Whether a prior art reference is enabling is a question of law based upon underlying factual findings." *SmithKline Beecham Corp. v. Apotex Corp.*, 403 F.3d 1331, 1337 (Fed. Cir. 2005). As MPEP 2121.01 states "In determining that quantum of prior art disclosure which is necessary to declare an applicant's invention 'not novel' or 'anticipated' within section 102, the stated test is whether a reference contains an 'enabling disclosure'. . .. *In re Hoeksema*, 399 F.2d 269, 158 USPQ 596 (CCPA 1968)." "A claimed invention cannot be anticipated by a prior art reference if the allegedly anticipatory disclosures cited as prior art are not enabled." *Amgen, Inc. v. Hoechst Marion Roussel, Inc.*, 314 F. 3d 1313, 1354 (Fed. Cir. 2003). To serve as an anticipating reference, the reference must enable the allegedly anticipated subject matter. *Elan Pharm. Inc. v. Mayo Found. For Med. Educ. & Research*, 346 F.3d 1051, 1054 (Fed. Cir. 2000). The challenged reference in *Elan* was an issued U.S. patent to Mullan, that like Fire here, broadly disclosed a biological method. The court there said

> The issue is not whether the teachings are an accurate compilation of the scientific state of the art at that time, and they are not challenged on that ground. The issue is whether his [Mullan's] teachings enabled a person of ordinary skill, without undue experimentation, to produce the desired transgenic mouse.

The court remanded the case for consideration of the relevant evidence, and the required application of the law to the facts of the case.

In a recent pronouncement relating to enablement of prior art references, the Federal Circuit favorably cited the above-quoted portions of both *Amgen* and *Elan*. *Rasmusson v. Smithkline Beecham Corp.*, 413 F. 3d 1318, 1325 (Fed. Cir. 2005).

On November 20, 2006 the Federal Circuit in *Impax Labs, Inc. v. Aventis Pharm.* ((Fed. Cir. 2006) slip opinion 05-1313 at 28) made clear that although neither proof of utility nor proof of efficacy for a fully disclosed compound are required, it is still necessary that the claimed invention must be described sufficiently to enable an ordinarily skilled artisan to carry out the invention. The court there declared that while anticipation does not require performance of the suggestions in a disclosure, the suggestions must be enabling to one of skill in the art. The court ultimately remanded the case to the district court for a full evaluation of the required factual determinations and legal analysis in connection with one of the asserted references. Finally, as the *Elan* court stated "an enablement determination is made retrospectively, i.e., by looking back to the filing date of the patent application and determining whether undue experimentation would have been required to make and use the claimed invention at that time."

The prosecution history in the instant case reflects that the Office acknowledges that extensive experimentation would have been required to for a skilled artisan armed only with the knowledge in the art and Fire's specification, to practice Applicant's invention. Applicant does not dispute the extent of experimentation required, but rather asserts that such experimentation would have been undue. Thus, the question is whether the experimentation required is undue, a question resolved with reference to the factors set forth in *In re Wands*, as has been discussed in prior responses. Applicant has submitted evidence throughout the prosecution to be considered in such analysis.

To summarize the facts and evidence of record, Applicant notes the following:

1. A declaration submitted by Applicant Alan Gewirtz provides evidence that a skilled artisan would not have been able to practice Applicant's claimed invention based on Fire's specification and the knowledge in the art at the time of Fire's filing. Evidence that Fire, a skilled artisan in the field of RNAi, admitted that the methods he disclosed could not be practiced in mammalian cells, or more specifically, human cells even after the Fire patent was filed, was also summarized in Dr. Gewirtz's declaration.

2. Fire's own work, published in *Trends in Genetics*, 15:358-363 (1999) (Exhibit 1), a peer-reviewed reference published after the filing date of the Fire patent, makes it clear that not only Fire, but skilled artisans generally, had no knowledge that RNAi could be practiced in mammals, and certainly did not believe the "simple protocols" used for invertebrates and plants would be successful in mammalian or human cells. Fire stated, for example:

> From a technical perspective, one could certainly hope that RNA-triggered silencing would exist in vertebrates: this would facilitate functional genomics and might allow medical applications involving targeted silencing of 'renegade' genes. Although this hope is not ruled out by any current data, the simple protocols used for invertebrate and plant systems are unlikely to be effective.

There is no reasonable interpretation of this publication that supports a position that the disclosure of the Fire patent enables the skilled artisan to practice the invention claimed by Applicant.

Fire thus admits that the method, the same method disclosed in his patent, and necessary to practice his invention, did not yet exist at the time of his filing, nor even at the time of publication of his subsequent article. Notwithstanding the content of Fire's claim, Fire himself never purported to teach the applicability of his method for mammalian cells, let alone considered it enabled for such purposes.

3. Another peer-reviewed publication, again Fire's own work, published with Montgomery (*Trends in Genetics* 14(7):255-257 (1998) (Exhibit 2) also shows the state of the art and provides compelling evidence that Fire's patent application could not have enabled the ordinarily skilled artisan to practice Applicant's claimed invention. The article contains a section entitled "Do RNA-interference mechanisms have counterparts outside of plant and nematodes?" Because the article did not purport to answer the question, by Fire's admission, the state of the art at the time was that it was not known whether RNAi even existed in mammals.

After reviewing the global antiviral response of mammalian cells to dsRNA, the PKR response, and the general translational arrest that was appreciated to occur in mammalian cells, the authors suggest that, because such responses can occur even where the dsRNA is taken up after being provided extracellularly, they probably evolved from responses to viral challenges. The authors conclude that

> Any gene-specific interference by dsRNA in PKR-proficient mammalian cells would be dependent on a transient lapse in the PKR response, or on a controlled level of dsRNA that was incapable of activating PKR.

In addition to being peer-reviewed, this paper is incorporated by reference in the Fire patent, and thus constitutes a teaching of the Fire patent itself, and as such, must be accorded proper weight. (see column 18, lines 42-46, and column 22, lines 5-8). Fire's patent specification itself then teaches the state of the art at the time, and that a transient lapse in PKR, or a dose incapable of activating PKR would be required to practice the method in mammalian cells. Yet, the specification provides no guidance on any doses that provide a "controlled level of dsRNA" incapable of activating PKR, yet are capable of inducing RNAi. Nor does Fire provide any guidance as to conditions, doses, or the like, that provide a "transient lapse in the PKR response."

4. The reference by Sharp (*Genes & Dev.* 13:139-141, 1999), provided by the examiner in the pending Office Action, is evidence of the state of the art after the filing date of the Fire patent. The Office Action cites Sharp for the proposition that "RNAi has been shown to work on specific genes in many organisms and concludes 'the RNAi phenomenon is likely to be a general mechanism for gene regulation and may be critical for many developmental and antiviral processes.'" The statements cited in the Office Action relating to Sharp are taken out of context, and their meaning is greatly extended. The statements were offered by Sharp in the introductory paragraph of the paper, immediately following a recitation of all the organisms in which dsRNA silencing of genes was known. The list included the invertebrates, *C. elegans*, *Trypansoma brucei* and *Drosophila*, and plants. Sharp's introductory statement must be viewed in the context of the entire paper, including the only paragraph in the three-page paper to address dsRNA in vertebrates. In that paragraph, Sharp concludes the article stating "[p]erhaps some aspect of the RNAi effect occurs or can be induced in mammalian cells."

As of its 1999 publication date, the peer-reviewed Sharp paper, taken as a whole, suggests that RNAi was an established phenomenon in invertebrates, and in plants dsRNA-mediated suppression of specific genes was known. But the paper also indicates that it was not known whether any aspect of RNAi occurs in, or can be induced in, mammalian cells.

Accordingly, the ordinarily skilled artisan could not have practiced Applicant's claimed methods based on Fire's patent and the state of knowledge existing in the art at the time of Fire's filing.

5. As discussed in Applicant's prior response, Kreutzer described the state of the art in his U.S. Patent Application Publication 2004/0175703, published September 9, 2004. Kreutzer's comments reflect a conclusion that protocols used for invertebrate and plant systems would not be effective for mammalian applications of dsRNA:

> WO 99/32619 (Fire *et al.*) discloses the use of a dsRNA of at least 25 nucleotides in length to inhibit the expression of a target gene in *C. elegans*. dsRNA has also been shown to degrade target RNA in other organisms, including plants (see, *e.g.*, WO 99/53050, Waterhouse et al.; and WO 99/6163-1, Heifetz *et al.*) and Drosophilia (see, *e.g.*, Yang, D., *et al., Curr. Biol.* (2000) 10:1191-1200). Despite successes in these organisms, until recently the general perception in the art has been that RNAi cannot be made to work in mammals. It was believed that protocols used for invertebrate and plant systems would not be effective in mammals due to the interferon response, which leads to an overall block to translation and the onset of apoptosis (see, *e.g.*, Wianny, F., *et al., Nature Cell Biol.* (2000) 2:70-75; Fire, A., *Trends Genet.* (1999) 15:358-363; and Tuschl, T., *et al., Genes Dev.* (1999) 13(24):3191-97). At least one group of scientists believed that RNAi could only be made to work in mammals if the PKR response could be neutralized or some way avoided, although no suggestions were given as to how this might be achieved (Fire, *Trends Genet* (1999), *supra*; and Montgomery and Fire, *Trends Genet.* (1998) 14:255-258). However, WO 00/44895 (Limmer) demonstrated for the first time that dsRNA can induce RNAi in mammalian cells, provided that the dsRNA meets certain structural requirements, including a defined length limitation.
>
> Kreutzer (see paragraph [0006])

Other of Kreutzer's patent applications have also been referenced as evidence of the state of the art during prosecution of this application. The pending Office Action appears to discount such disclosure, concluding "[i]t is important to note that these comments come from a competitor of Fire *et al.*, in a patent application, which therefore does not carry the same weight as if they appeared in a peer-reviewed journal article."

The teachings of Kreutzer constitute part of the prior art (see, e.g., MPEP 2123) and must be given fair weight for their evidentiary value. The quoted segment is part of the background section of the Kreutzer specification, and while not a peer-reviewed publication, the section is amply referenced with citations to the peer-reviewed literature that accurately reflect the state of

the art at the time. Applicant will gladly supply each of the peer-reviewed references cited by Kreutzer, although they are predominantly already of record in the instant prosecution history. Further, Kreutzer's work has been asserted by the Examiner against the instant claims. Kreutzer's teachings cannot, on the one hand, be asserted as enabling anticipatory art and, on the other hand, be disparaged by the Examiner as mere puffing of Fire's competitor.

6. A peer-reviewed paper by Paddison *et al., Proc. Nat'l Acad. Sci.* 99(3):1443-1448 (Feb. 5, 2002) (Exhibit 3) has been cited by the Applicant during this prosecution as further evidence that the Fire patent does not teach each and every element of the claimed invention, or in the alternative, does not enable the skilled artisan to practice the method in its entirety. Paddison teaches that "[i]t has become clear that dsRNA-induced silencing phenomena are present in evolutionarily diverse organisms including plants, fungi, and metazoans." Citing the work of Wianny *et al.* (*Nat. Cell Biol.* 2:70-75, 2000), as well as that of Svoboda *et al.* (*Development (Cambridge UK)* 127: 4147-4156, 2000), Paddison states in his discussion that [t]he first indication that this (RNAi) response might also extend to mammals came from the observation that injection of dsRNAs into early mouse embryos induced sequence specific silencing. Applicant notes this article appeared in one of the most prestigious scientific journals in the U.S.. Applicant further notes that the Wianni *et al.* and Svoboda *et al.* papers on gene silencing in mouse embryos published three years after the filing of the Fire patent.

7. Wianny *et al.* (Exhibit 4) and Svoboda *et al.*(Exhibit 5), both peer-reviewed research articles, provide further evidence that the Fire patent did not have sufficient disclosure to enable the ordinarily skilled artisan to practice the instant claims. Wianny *et al.* flatly state that "[s]o far, there has been no report that RNAi can be used in mammals. Moreover there are several indications of potential limitations to its function in the group of animals."

Applicant respectfully submits that evidence has been presented that is more than adequate to overcome the presumption of enablement of the reference. Applicant respectfully submits that the evidence of record establishes that the Fire patent would not have enabled any skilled artisan of ordinary skill to practice Applicant's claimed method without undue experimentation. Not only would the amount of experimentation been excessive, Fire does not

provide any guidance, for example, on how to select an amenable cell, what gene to target, what dose of dsRNA to use, under what conditions, for what length of time, to accomplish a measurable reduction, without creating a generalized or nonspecific response (e.g. a "panic" response) to the dsRNA. Though Fire provides recitations of cell types and ranges of doses, the skilled artisan cannot "at once envisage" what to do, or how to do it. Fire provides no working examples with any vertebrate cells, nor with any mammalian cells, let alone human cells. The nature of Applicant's invention is both complex and important, except in hindsight wherein it appears simple and merely repetitive. The level of skill in the art is very high – the ordinarily skilled artisan is at least a Ph.D. and probably one with post-doctoral level biotechnology experience, and yet the state of the art at the time was such that no one even recognized that dsRNA-induced gene silencing was attainable in *any* vertebrate system, especially mammalian. The evidence of record is replete with statements doubting the existence of RNAi in mammalian systems, and questioning the applicability of invertebrate or plant methods to mammalian cells. Thus, the art of gene silencing, at the time, demonstrated a lack of predictability with regard to success in mammalian systems. The art also plainly established that mechanisms of dsRNA-induced gene silencing were not understood completely in any organism, including what the responsible cellular systems might be, what enzymes or receptors might be involved, and what function these factors might play in the organism. The art failed to address other issues concerning gene silencing such as how systems in plants, single-celled organisms or lower animals might relate to each other, or to more complicated systems.

Applicant's disclosure is directed to mammalian cells. The instant claims are even narrower, directed solely to human cells. In view of the evidence discussed above, Applicant respectfully submits that the level of experimentation required to enable the disclosure of the Fire specification for mammalian cells was not only extensive, but was plainly undue based on the analysis under *Wands*.

The facts of this case are very similar to those in *In re Goodman* (11 F. 3d 1046 (Fed. Cir. 1993). There, the applicant had claimed a method of manufacturing mammalian peptides in plant cells using *Agrobacterium*-mediated transformation. Goodman's specification *exemplified* only dicots. The Federal Circuit upheld the Board's rejection of the claims for lack of enablement because the specification did not contain sufficient information to enable the scope

of the claims beyond dicots. The court noted, for example, that the production of peptides in monocot plants involves extensive problems unaddressed by Goodman's specification.

While Goodman deals with enablement of claims under § 112, and not enablement of a reference under § 102, the facts are remarkably similar to the present case. The disclosure of Fire, like that of Goodman, fails to provide the details necessary to practice the invention in a category of hosts (mammalian cells), in the fact of art-recognized doubts of the invention's applicability to those hosts. The enablement issue here must be assessed with reference to an analysis under *Wands* to determine whether the skilled artisan could practice the instantly claimed invention using only the Fire reference and the knowledge in the art at the time.

The *Goodman* court was persuaded to uphold the 35 U.S.C. § 112, first paragraph rejection in view of the unpredictability of the art, as reflected in an article stating:

> It has been widely considered that monocotyledonous plants, including the commercially important crop plants of the *Gramineae* family, are insensitive to [Ti plasmid transformation] and thus are not candidates for the use of this gene transfer system. Two more recent reports have modified this opinion to some extent. . .

Id. at 1051.

The court concluded that this article, coupled with the "hedgings" in other references, showed unpredictability in the art when Goodman filed his application in 1985.

Significantly for the case under examination here, another paper published by Goodman himself two years after filing, where he had reported only limited success, was cited by the court. Goodman's paper stated that "[i]n plants, viral-based vectors are not likely to stably transform plant cells because integration of viral genes into plant chromosomes has not been detected." *Id.* at 1051-52.

As a result of the unpredictability of the art and Goodman's admission, the Federal Circuit held:

> Thus, on Goodman's 1985 filing date, the record shows no reliable gene transformation method for use with monocot plants. Each of the methods for monocot plants was fraught with unpredictability. The teachings in the specification do not cure this unpredictability. The record shows that practicing a gene transformation method for all monocot plants, if possible at all in 1985, would have required extensive experimentation that would preclude patentability. [**16] See White Consol. Indus. Inc. v. Vega Servo-Control, Inc., 713 F.2d 788, 218 U.S.P.Q. (BNA) 961 (Fed. Cir. 1983). . . .

> The record, *especially Goodman's own article*, shows the need for extensive experimentation to practice the claimed method for just a few plants, let alone all plant cells as broadly claimed in the application.

Id. at 1052 (emphasis added).

The parallels with Applicant's case are clear. The Fire patent would not have enabled a skilled practitioner to practice Applicant's claimed invention without undue experimentation.

The Office Action also appears to conclude that Fire's patent is a pioneering patent and that somehow influences the enablement analysis. Assuming, *arguendo*, that Fire is "pioneering", the examiner is invited to provide support for the notion that a patent's status as a "pioneering patent" impacts the enablement analysis. In fact, such a premise is contrary to the law, particularly as it relates to the § 102 enablement question at issue here. The Federal Circuit has been completely clear that such "pioneering" status of a patented invention does not entitle the patent to a lower standard of enablement. See *Plant Genetic Systems N.V. v. DeKalb Genetics Corp.*, 315 F.3d 1335, 1340-41 (Fed. Cir. 2003).

Applicant respectfully requests reconsideration, in view of the evidence now before the examiner, and withdrawal of the rejection under 35 U.S.C. § 102(b) with respect to the Fire patent in accordance therewith.

As to the burden of proof, the Office Action suggests that Applicants are "inappropriately" shifting the burden of proof to the examiner. Applicants respectfully, but emphatically, submit, as detailed above, that any initial presumption of enablement of the Fire reference has been overcome by the evidence provided. In that regard, the burden is now properly on the Office to make any contrary evidence of record, weigh the evidence, and make a determination based on that evidence, without any presumption that Fire's specification is enabling vis-à-vis Applicant's claims.

Finally, contrary to the assertions on the Office Action, Applicant's disclosure provides something new that the public was not previously in possession of, notwithstanding Fire's disclosure, namely methods for applying RNAi in human cells. If the assertion is correct in the Office Action, that "literally thousands" of post-filing reference show that Fire's invention works for human cells, it should not be a problem for the Examiner to find and make of record references that are post-filing for Fire and pre-filing for Applicant that support that notion.

Applicant respectfully requests reconsideration in view of the foregoing, Withdrawal of the rejection under 35 U.S.C. § 102(b) is appropriate in view of the evidence that either Fire does teach each and every limitation of Applicant's claims, or that the Fire patent would not have enabled the ordinarily skilled artisan to practice Applicant's invention without undue experimentation.

Fire does not teach each and every limitation of newly added claim 28 within the meaning of 35 U.S.C. § 102.

Applicant has submitted herewith new independent claim 28. During extensive prosecution, Applicant has set forth evidence and argument to establish why Fire does not anticipate claim 1 and claims dependent thereon – it does not teach each and every step of the claimed methods, and even if it is somehow construed to do so, it would not have enabled a skilled artisan to practice Applicant's claimed invention at the time of Fire's filing. Applicant respectfully further submits that new claim 28, while not currently rejected, is not anticipated by the Fire patent.

What a prior art reference actually discloses for purposes of anticipation analysis is a factual determination. *Tegal Corp. v. Tokyo Electron Am., Inc.*, 257 F.3d 1331, 1338–39 (Fed. Cir. 2001). The law of anticipation requires that the same invention, with all the limitations of the claims, existed in the prior art. *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920-21 (Fed. Cir. 1989) ("anticipation" requires that the identical invention is described in a single prior art reference) MPEP 2131.01 states "The identical invention must be shown in as complete detail as is contained in the ... claim." *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989).

New claim 28, and claims dependent thereon, are generally directed to methods for disrupting expression of a target gene level in a human cell. The claimed method comprises the steps of:

(a) selecting a human cell expressing the target gene;

(b) preparing a double-stranded RNA (dsRNA) consisting essentially of a first strand homologous to the target gene, and a second strand complementary to the first strand;

(c) exposing the human cell to the dsRNA in a reaction mixture *in vitro*, under conditions permitting the dsRNA to enter the cell; and

(d) incubating the reaction mixture for a time sufficient to allow the initiation of RNA interference;

thereby disrupting expression of a target gene level.

The method optionally comprises the further steps of:

(e) measuring the expression of the target gene in the exposed cell; and

(f) comparing the expression of the target gene in the exposed cell to that of a control cell that was not exposed to the dsRNA,

wherein a decrease in expression of the target gene in the exposed cell relative to that of the control is indicative of disruption of the expression of the target gene.

Here, in the rejection to claim 1 and claims dependent thereon, the Office Action asserts that Fire "teaches" RNAi in mammalian, and even human, cells, including a vast litany of cancerous cells. While Applicant respectfully disputes this as a factual matter, Applicant concedes that there are recitations or laundry lists in Fire which may state that mammalian or human cells may be used. However, such recitations do not rise the level of a "teaching", as they are merely boilerplate and did not, at the time of Fire's filing, provide a skilled artisan with what was needed to practice Applicant's now claimed methods in mammalian cells. Fire's disclosure purports to treat or prevent a wide variety of diseases using RNAi, including cancer and HIV, and includes ameliorating any symptom associated with the disease, or clinical indication of the pathology. Applicant asserts that none of these statements rise to the level of "teachings" because they do not enable a skilled artisan to practice that which is allegedly "taught" – thus they are truly empty recitations, particularly with respect to the patentability of Applicant's claim 28 and claims dependent thereon.

For example, Fire does not teach the step of selecting the human cell. Fire largely exemplifies treating the whole organisms, yet acknowledges that certain cells are nonresponsive (Fire, e.g., columns 16 and 17). Elsewhere, Fire states that

> Although sensitivity to dsRNA-triggered PTGS (post translational gene silencing) appears to be the rule rather than an exception, there might be some target RNAs that partially or fully resist PTGS. Similarly some tissues might be partially resistant to the effect (including some parts of the *C. elegans* nervous system; J. Fleenor and A. Fire, unpublished).

(See section "Can any RNA be a target of PTGS?" on page 360; Fire, TIGS 15: 358-63 (1999) (Exhibit 1)). The skilled artisan received no guidance and would have had no basis, at the time, for selecting a human cell in which the method would work.

Further, Fire does not teach the conditions required in step (c), nor the time required in step (d) of claim 28. Fire provides two examples of methods for introducing a dsRNA into the organism, namely injecting *C. elegans* adults, or bathing the larvae. Fire recites that other methods may be used to expose an organism or a cell to a dsRNA, however, Fire provides no additional guidance required by the skilled artisan to practice the method in human cells.

For example, for injection experiments in *C. elegans*, Fire used 0.5 to 1 x 10^6 molecules (less than about 1 pg, based on Applicant's calculation), while for the bathing experiments he used concentrations of 1 mg/ml and immersed intact *C. elegans* larvae therein (see Fire at column 19, lines 26-28 (injections); and at column 18, lines 20-24 (bathing)). Neither of these dosages would have enabled the skilled artisan, at the time of Fire's filing, to achieve success in mammalian cells in accordance with Applicant's methods, as they do not provide the required conditions. As is shown in Applicant's specification, concentrations of about 150-350 μg of dsRNA /ml of reaction mixture (e.g. media) were useful. (See Applicant's specification at pages 13-14, particular lines 16-17 on page 13). Fire purports to teach other broad ranges of doses, but provides no guidance on how to select a suitable dose for different cells or cell types.

In addition, Fire exemplifies bathing intact *C. elegans* larvae for 24 hours (see column 18, lines 20-24). Fire does not teach, and gives no guidance on any other times of exposing *C. elegans* larvae, or *C. elegans* cells, or any other cells to dsRNA. Such times would not have been effective and the skilled artisan would not have been able to practice the claimed methods. As is shown in Applicant's specification, in one experiment, the Applicant added dsRNA homologous to a portion of the c-kit gene to mammalian cells (specifically two different types of human cells) and incubated the cells with dsRNA for 1-4 days. Applicant found no effect on c-kit expression until day 3 of incubation with the dsRNA. (See, e.g., Applicant's specification at page 14, Table 1). Assuming for the sake of argument that the skilled artisan selected an amenable cell, he still would not have sufficient guidance from Fire's specification to determine a suitable dose of dsRNA or how long to incubate the cells to accomplish RNAi.

Thus, Fire does not teach each and every limitation of the invention claimed in claim 28 – he does not teach selecting a human cell, he does not teach the conditions for exposing the cell, e.g. he does not teach any dose of dsRNA suitable for *any* mammalian or human cell, or any satisfactory incubating time in accordance with Applicant's claimed methods. Contrary to the assertions in the Office Action, these are manipulations that completely distinguish the instant

claims from the actual and purported teachings of Fire. Further, contrary to the assertions in the Office Action, had Fire tried his methods with human cells – he would have failed for the reasons stated. The same invention is simply not disclosed in the same detail, as is required under 35 U.S.C. § 102.

The Claims are Patentable Over Fire in view of Gewirtz, Kreutzer, and Sharp.

Claims 23-27 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Fire *et al.* ("Fire"), in view of Gewirtz *et al.* (WO 92/19252) ("Gewirtz"), Kreutzer *et al.* (WO 00/44895) ("Kreutzer"), and Sharp (Genes and Dev. 13:139-141 (1999)). Applicant respectfully traverses the rejection.

The Office Action asserts that Fire discloses a method for inhibiting expression of a target gene using dsRNA to induce RNAi in a cell *in vitro*, wherein the cell is from an animal. Fire *et al.* allegedly also dislcose that the cell can be from a human, and may be "immortalized or transformed, or the like," and that the method can be used for treatment or development of cancers of any type, including solid tumors, sarcomas, and leukemias. Fire *et al.* allegedly further disclose that their method has advantages over antisense approaches. The Office Action acknowledges that Fire does not teach the sequence of the c-kit oncogene. However, Gewirtz is cited to show the c-kit cDNA sequence was known in 1987. Kreutzer is cited as allegedly teaching methods specifically for disrupting mammalian target gene *in vitro*, by administering a small double-stranded RNA homologous to the target gene to induce RNAi wherein the targets are oncogenes, and the cells are malignant, and wherein the RNA is formulated as part of a pharmaceutical formulation, or targets a human disease or disorder. Sharp is added as a general reference allegedly supporting the notion that RNAi is likely to be a general mechanism for gene regulation and may be critical for many developmental and antiviral processes.

The Office Action alleges that it would have been *prima facie* obvious to the ordinarily skilled artisan to substitute an siRNA oligonucleotide in place of the antisense oligonucleotide in an *in vitro* method for inhibiting the expression of c-kit (as taught by Gewirtz) wherein the dsRNA was in a pharmaceutical composition because dsRNA used to inhibit gene expression has advantages over antisense in the stability of the material to be delivered (as taught by Fire and

Kreutzer), and because dsRNA can be used to initiate RNAi *in vitro* by targeting oncogenes in human cells (as taught by Fire or Kreutzer).

The Office Action alleges that a motivation for such a combination would have come from Gewirtz's success in inhibiting c-kit in human leukemia cells, and because of the aforemention advantages of stability. An expectation of success in practicing the method allegedly would have stemmed from Gewirtz's success in inhibiting c-kit in human cells, and because Fire *et al.* teach that dsRNA can be used to initiate RNAi, and because of advantages in the stability of the material to be delivered. Sharp's disclosure that RNAi is likely to be a general mechanism for gene regulation and may be critical for many developmental and antiviral processes is added as further reason for an expectation of success.

Applicant respectfully asserts that the *prima facie* case rises, at most, to an obvious to try standard, which is insufficient as a matter of law. Further, there would be no expectation of success as detailed below. As discussed above, the amendments to claim 22 entitle those claims to the earlier priority date, making Kreutzer unavailable as prior art under 35 U.S.C. § 102, and therefore equally unavailable under 35 U.S.C. § 103. With regard to Fire, as discussed above, the disclosure in Montgomery and Fire, which is incorporated by reference into Fire itself, and thus form a part of the Fire specification, directly teach away from the use of mammalian cells at the time of the filing of Fire's application. Fire and Montgomery unequivocally question whether mammalian cells have the machinery for dsRNA-induced gene silencing. "Any gene-specific interference by dsRNA in PKY-proficient mammalian cells would be dependent on a transient lapse in the PKR response, or on a controlled level of dsRNA that was incapable of activating PKR." Such disclosure undercuts the disclosure of the Fire patent and teaches away from Applicant's invention. The skilled artisan reading Fire and Gewirtz together at the time, would not have been motivated to combine, and even if he were motivated to try to combine the methods, he would not have had any expectation of success in dsRNA-induced silencing of c-kit in mammalian cells because the skilled artisan would have had to first overcome the problem of the PKR response.

The teachings of Sharp do not cure the deficiencies noted above in the other references. The cited portion of Sharp is clearly out of context. It follows a discussion of all those organisms known to possess RNAi responses – i.e. invertebrates and plants. Applicant concedes that dsRNA could have been appreciated as a general mechanism for gene regulation among

invertebrates and plants based, for example, on Fire and Sharp. That said, the skilled artisan knew nothing of RNAi in vertebrate or mammalian systems. Taken as whole, Sharp would actually also lead a skilled artisan away from the asserted combination, or at best indicate the combination may be "obvious to try". As discussed in detail above, Sharp is predominantly about dsRNA in invertebrates and plants. As to dsRNA in vertebrates, Sharp merely speculates: "Perhaps some aspect of the RNAi effect occurs or can be induced in mammalian cells." Since the skilled artisan at the time did not know or have any reason to believe that mammalian cells had a RNAi response, there could not have been any expectation of success even if the artisan were motivated to try the asserted combination.

Finally, even if the skilled artisan attempted to combine Fire and Gewirtz in a human cell, he would not have been successful because Fire provides no guidance on the concentration or length of time to obtain an RNAi effect in such cells.

In accordance with the foregoing, Applicant respectfully requests reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a).

Conclusion

The claims are in condition for allowance. Favorable reconsideration and a notice to that effect are earnestly requested. The Examiner is invited to contact the undersigned representative to resolve any outstanding minor issues prior to allowance.

Respectfully submitted,

ALAN GEWIRTZ

BY _____

DANIEL A. MONACO
Attorney Registration No.: 30,480

DRINKER BIDDLE & REATH LLP
One Logan Square, 18th and Cherry Streets
Philadelphia, PA 19103
(215) 988-3312 *direct*
(215) 988-2757 *facsimile*

Attorney for Applicant